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**Please find below and/or attached an Office communication concerning this application or proceeding.**

The time period for reply, if any, is set in the attached communication.

## Office Action Summary

Application No.

10/812,646

Applicant(s)

LIEW, CHOONG-CHIN

Examiner

Juliet C. Switzer

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

### Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

### Status

- 1) ☒ Responsive to communication(s) filed on 23 October 2007.
- 2a) ☒ This action is **FINAL**. 2b) ☐ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

### Disposition of Claims

- 4) ☒ Claim(s) 52-54 and 56-80 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 52-54 and 56-79 is/are rejected.
- 7) ☒ Claim(s) 80 is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

### Application Papers

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
- Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
- Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

### Priority under 35 U.S.C. § 119

- 12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☐ Some \* c) ☐ None of:
- ☐ Certified copies of the priority documents have been received.
  - ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  - ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

### Attachment(s)

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☐ Information Disclosure Statement(s) (PTO/SB/08)  
Paper No(s)/Mail Date \_\_\_\_\_
- 4) ☒ Interview Summary (PTO-413)  
Paper No(s)/Mail Date 20071205
- 5) ☐ Notice of Informal Patent Application
- 6) ☐ Other: \_\_\_\_\_

### DETAILED ACTION

1. This action is written in response to applicant's correspondence received 10/23/07.

Claims 52-54 and 56-57 have been amended, claims 49-51 and 55 have been canceled, and claims 58-80 have been added. Claims 52-54, and 56-80 are pending are examined herein.

Applicant's amendments and arguments have been thoroughly reviewed, but are not persuasive to place the claims in condition for allowance for the reasons that follow. Any rejections not reiterated in this action have been withdrawn. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action. **This action is FINAL.**

#### *Claim Objections*

2. Claim 80 is objected to under 37 CFR 1.75(c) as being in improper form because a multiple dependent claim cannot depend from a previous multiple dependent claim. See MPEP § 608.01(n). Accordingly, the claim 80 has not been further treated on the merits.

#### *Claim Rejections - 35 USC § 112*

3. Claims 64, 65, 67, 69, 71, 72, 73, 74, 75, 77, 78 and 79 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a rejection for new matter.

Claim 64 appears to have new matter. The specification does not provide basis for a claim which broadly states that any time a test subject's RNA expression of CLC is "higher" than

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the expression of healthy control subjects that the subject is a candidate for having schizophrenia. Regarding the expression of CLC, the specification provides only one very specific teaching, while this claim encompasses a broad genus of "higher" expression values. Table 3Y teaches that the ratio of expression in schizophrenic samples relative to control samples is 2.25, indicating that in the tested samples, CLC was expressed, on average at a 2.25 times higher level in schizophrenic patients versus healthy controls. The specification teaches that the samples included 4 patients with Schizophrenia and six "control" individuals. Table 3Y teaches that this result is significant  $p=0.0212$ . The specification further provides example 51 which compares gene expression in patients having schizophrenia versus patients having manic depression syndrome. The specification teaches that 294 genes were identified as being differentially expressed, and regarding the instant claims, table 3AC provides a list of these genes (Example 51). CLC is not among the genes, suggesting that this gene is not differentially expressed in Schizophrenic patients versus patients with manic depression syndrome. Thus, the broad statement in the claim regarding classifying the subject as a candidate for schizophrenia if the RNA level "is higher" appears to be new matter. Claim 79 also recites new matter for similar concerns, and additionally for the recitation that the CLC expression "is statistically lower relative to said levels in said subjects classified as having schizophrenia."

Claim 65 appears to have new matter. The specification does not provide basis for the range "at least two times higher than that of control subjects." As discussed, the specification teaches only a single example where there was an observed ratio of 2.25 between patients having schizophrenia and healthy controls. There is no value for the range limit 2 nor for the range of values higher than 2.25.

Claim 67 appears to have new matter. The specification does not provide basis for the particular combination of observations required for the claim, namely that CCL expression in a test subject is "at least 2 times higher" with a "p value of  $<0.05$ ." The limitation "at least 2 times higher" has been previously discussed. Further, "with a p value of  $<0.05$ " does not appear to have basis in the context of differential expression of CLC in a test subject versus healthy subjects. Claim 69 also includes this recitation and is rejected for having the same new matter. In a similar fashion, claim 75 is rejected for new matter.

In claims 71, 72, 73, 74, and 77, the limitation that the blood samples "comprises leukocytes which have not been fractionated into cell types" is new matter. Such a recitation includes, for example, testing a blood sample where the red blood cells and the white blood cells have been separated, and also includes, the testing of whole blood RNA. There is clearly basis for the latter, but not the former. Applicant asserts in the remarks that this claim limitation finds clear support in the specification, including figure 5C which shows standardized fractions of leukocytes. However, these are not leukocytes that have not been fractionated into cell types, as they have clearly been fractionated into cell types. While RNA levels have been determined in each of the fractions, this is not basis for the negative limitation "have not been fractionated into cell types." There is no discussion or example in the specification of the testing of RNA in blood samples which comprise leukocytes which have not been fractionated into cell types. Applicant has attempted to present a claim which excludes a particular process step from a method (that is, fractionating the leukocytes) and then provides basis for the exclusion of the step in a method where the opposite occurred. This is not sufficient basis for the claim limitation because there is nothing in the specification that suggests applicant contemplated the exclusion of a step of

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fractionating leukocytes into cell types. Therefore, the claims are rejected for having new matter.

All claims which depend from the specifically discussed claims are rejected for having new matter because of their dependency from the specifically enumerated claims.

4. Claims 52-54, 56-74 and 79 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

#### **Nature of the invention**

Claim 58 is drawn to a method for detecting expression of a gene encoding a Charcot-Leyden crystal protein (CLK1) in a human "test subject." Claims which depend from claim 58 set forth that the detected expression is quantified and compared to quantified level of control RNA encoded by said gene in blood samples of control subjects. Listed control subjects include healthy subjects, subjects having schizophrenia and subjects that do not have schizophrenia. Further dependent claims set forth steps of classifying or identifying the test subject as being a candidate for having schizophrenia depending on the outcome of the comparing steps. Thus, it is clear that the intended use of claim 58 and those that depend from claim 58 is for classifying or identifying the test subject as being a candidate for having schizophrenia.

Independent claim 69 sets forth a method for screening a human test subject for having schizophrenia and includes similar detection, quantification, and comparing steps, reciting that a test subject is a candidate for having schizophrenia if said level of RNA encoded by said gene in said blood sample of the test subject is "at least 2 times higher" than that of said healthy control

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subjects with a p value  $<0.05$ . Claim 70 is similar, but recites that the subject is a candidate for having schizophrenia if the level of RNA encoded by said gene is 2.25 times higher than that of said control subjects classified as healthy subjects with a p value equal to 0.0212.

The nature of the invention requires the knowledge of a reliable relationship between CLC expression in blood and the presence of schizophrenia.

In claim 79, the invention is drawn to a method a method for classifying CLC gene expression in a human, and sets forth steps of quantifying a level of RNA encoded by a CLC gene, comparing that level to a level of RNA found in blood samples from control subjects having schizophrenia and also comparing it to control subjects who are healthy. The independent claim states that based on particular determinations, the classification of CLC gene expression results either with that of said subjects having schizophrenia or with that of subjects who are healthy.

The nature of the invention requires the knowledge of a reliable association between CLC expression and the ability to classify a particular individual's expression with the expression of subjects having schizophrenia or not having schizophrenia, and further, the use of this method requires that there is an underlying assumption that this classification is meaningful. Reading the claims in light of the specification it is clear that applicant intends to use such a classification method in order to provide a tool that is used as part of a diagnostic process, and such a use requires the knowledge of a reliable association underlying the classification. Further, the practice of the invention requires an understanding of how the presence of schizophrenia effects the level of CLC expression in human blood in patients having schizophrenia versus patients that do not have schizophrenia but may have some other disorders.

### **Scope of the claims**

Many aspects of the claims remain quite broad.

In some claims the health status of the control individuals is entirely undefined, and encompass subjects with schizophrenia, healthy patients, patients with some other disease, such as depression or rheumatoid arthritis or multiple sclerosis.

Many claims recite that a difference is identified but do not require that the difference is statistically significant at any particular level, and so, any level of difference observed can result in classifying the test subject as a candidate for disease. These claims do not recite a level of statistical significance that is required to be reached, and so, the claims remain quite broad since no particular level is required, and the claims even encompass using different levels of statistical significance for different comparisons. The phrase "statistically significant" describes a mathematical measure of difference between groups, not a particular level of difference which is acceptable. There is no universally accepted level of "statistically significant."

Claim 58 is representative of the narrowest claims set forth in the instant claim set that sets forth relationships that are supported by the data in the specification. This claim specifically defines the control population as healthy subjects and sets forth a very particular ratio of gene expression in the test subject relative the healthy control subjects.

### **Teachings in the Specification/Examples**

Regarding schizophrenia, the specification provides example 27 wherein gene expression profiles of blood samples from individuals having schizophrenia were compared with normal individuals, that is healthy patients. The specification teaches that 1,952 genes were identified as

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being differentially expressed, and regarding the instant claims, table 3Y provides a list of these genes (Example 27). CLC is among the genes.

Table 3Y teaches that the ratio of expression in schizophrenic samples relative to control samples is 2.25, indicating that in the tested samples, CLC was expressed, on average at a 2.25 times higher level in schizophrenic patients versus healthy controls. The specification teaches that the samples included 4 patients with Schizophrenia and six "control" individuals. Table 3Y teaches that this result is significant  $p=0.0212$ .

The specification further provides example 51 which compares gene expression in patients having schizophrenia versus patients having manic depression syndrome. The specification teaches that 294 genes were identified as being differentially expressed, and regarding the instant claims, table 3AC provides a list of these genes (Example 51). CLC is not among the genes, suggesting that this gene is not differentially expressed in Schizophrenic patients versus patients with manic depression syndrome.

The claims are inclusive of claims which classify a subject as "a candidate for schizophrenia" based on any observation of any "higher" CLC expression relative to healthy controls, yet the specification teaches that 2.25 fold difference in expression was observed.

Claim 54 is limited to a case where the control subjects do not have schizophrenia, but they could still have any other possible disease or condition. For example, the claims are inclusive of control subjects that have manic depression syndrome. For this embodiment of the claims, the specification does not provide information about an essential aspect of the invention, namely, evidence that there is a difference in expression of the CLC gene between these two populations.

Furthermore, though the specification teaches that this gene is differentially expressed in schizophrenia patients versus healthy patients, the specification teaches this is true for thousands of genes. There is no guidance or analysis of data in the specification to suggest that this gene in particular is sufficient to classify a patient as a candidate for schizophrenia, as instant claimed. This information is essential to understanding and practicing the claimed invention because it is critical to knowing how to interpret a particular comparison result.

#### **State of the Prior Art and Level of Unpredictability**

Observing differences in expression between two populations is a highly unpredictable endeavor. While the instant specification teaches that CLC is differentially expressed in a population of schizophrenia patients versus control subjects, the specification does not establish that any particular level of expression of CLC (relative level or raw level) is sufficient to reliably classify expression of a test subject with that of subjects having schizophrenia or to reliably identify a patient as a candidate for having schizophrenia.

Fjaerli et al. teach that CLC is downregulated in whole blood of infants hospitalized with respiratory syncytial virus. This exemplifies that it is highly unpredictable whether or not one can conclude, simply from a blood sample of a test patient, that schizophrenia is present, since differential expression versus a control could indicate some other disorder or phenotype is present, whether that is respiratory syncytial virus, manic depressive disorder or some other disease which has not yet been analyzed.

Iwamoto et al. teach that expression profiling in psychiatric fields have been notoriously discordant, with different studies often reporting conflicting gene expression data (The Neuroscientist, Vol. 12, Number 4, 2006, pages 349-361; Abstract and page 351). Tsuang et al.

undertake an analysis that is very similar to the one in the instant specification, with one major difference being that their sample size is larger. Regarding their results, Tsuang et al. caution that the results must be interpreted with caution given several limitations including small sample size, the fact that the findings are not replicated in a separate cohort and results “may represent chance findings and type-I inferential errors,” and that the patients tested were all on drugs that were not accounted for in the analysis (American Journal of Medical Genetics, Part B (Neuropsychiatric Genetics) 133B:1-5(2005)). All of these cautions set forth by Tsuang et al. appear to be equally or more relevant to the study set forth in the instant application. Vawter et al. teach that there is lack of consistency in the study of genes differentially expressed in schizophrenia which might be related to etiological and genetic heterogeneity of the illness (p. 42, Vawter et al. Schizophrenia Research, Vol. 67, pages 41-52, 2004). All of these taken together underscore and highlight the very unpredictable nature of this technology area.

Furthermore, although CLC was not observed to be differentially expressed in any of the other examples in this specification, it is unknown and unpredictable whether it would be expressed in the blood of patients having other mental illnesses or any other diseases which were not tested in the instant specification or diseases which were tested in the instant specification but in a different population of test subjects, and whether this expression would be different from levels of expression in healthy controls. It is unpredictable whether the gene is differentially expressed, for example, in patients having manic depression disorder versus healthy controls, and if it is, how this relates to the difference in expression between patients with schizophrenia and manic depression disorder. A method for classifying subjects or for screening subjects as a candidate for schizophrenia which relies on a comparison between expression in the blood of a

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test subject and control subjects requires the knowledge of this information in order to reliably make suggestions or drawn conclusions about the presence of schizophrenia, as set forth in the claims.

The instant specification has not established that all difference, no matter the magnitude nor the direction, relative to any control subjects or even relative to a healthy control subject is sufficient to conclude that a patient is a candidate for schizophrenia or that their expression level should be “classified” with patients having schizophrenia. Furthermore, the specification has not shown that all expression at a level statistically the same as that observed in a population of patients having schizophrenia is sufficient to conclude that schizophrenia is present. In fact, it is unclear if this is a fair conclusion given the fact that CLC is not differentially expressed in patients having schizophrenia versus manic depression syndrome- the same expression might indicate the presence of manic depression syndrome. It is entirely unpredictable if this is also the case with other diseases. It is not known under what circumstances the result observed in the instantly examined control and test populations would be repeatable, as the results have not been validated. All of these inquiries are particularly important in this case since the claims suggest or explicitly recite the intended use of classifying individuals and their expression levels.

Further, some of the claims of the instant application set forth the comparison of the gene expression in a single individual versus as few as two other individuals, and they set forth that a comparing gene expression between the two is useful to suggest the presence schizophrenia. Neither the specification nor the claims set forth a threshold of difference between an individual’s expression and the control expression of CLC in the blood that would be sufficient to conclude that the difference in gene expression between a test individual and any type control

group is sufficient to conclude that the test subject is a candidate for the recited schizophrenia. Because some of the claims encompass any level of altered gene expression, it is relevant to point out that the art of Cheung et al (2003) teaches that there is natural variation in gene expression among different individuals. The reference teaches an assessment of natural variation of gene expression in lymphoblastoid cells in humans, and analyzes the variation of expression data among individuals and within individuals (replicates) (p.422, last paragraph; Fig 1). The data indicates that, for example, expression of ACTG2 in 35 individuals varied by a factor of 17; and that in expression of the 40 genes with the highest variance ratios, the highest and lowest values differed by a factor of 2.4 or greater (Fig 3). It is thus unpredictable as to whether or not any level of altered gene expression is indicative of a schizophrenia or the absence of schizophrenia.

The unpredictability of correlating gene expression level to any phenotypic quality is taught in the post-filing art of Wu (2001). Wu teaches that gene expression data, such as microarray data, must be interpreted in the context of other biological knowledge, involving various types of 'post genomics' informatics, including gene networks, gene pathways, and gene ontologies (p.53, left col.). The reference indicates that many factors may be influential to the outcome of data analysis, and teaches that expression data can be interpreted in many ways. The conclusions that can be drawn from a given set of data depend heavily on the particular choice of data analysis. Much of the data analysis depends on such low-level considerations as normalization and such basic assumptions as normality (p.63 - Discussion). The art of Newton et al (2001) further teaches the difficulty in applying gene expression results. Newton et al. teaches that a basic statistical problem is determining when the measured differential expression

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is likely to reflect a real biological shift in gene expression, and replication of data is critical to validation (p.38, third full paragraph). There is no replication of data in the instant specification.

### **Quantity of Experimentation**

The instant specification does not provide enabling support for the practice of a single embodiment within the claimed invention. In particular, the specification does not provide adequate guidance to appraise one of ordinary skill in the art as to what levels of CLC gene expression must be observed to successfully conclude that schizophrenia is present. Although the specification teaches there are differences in CLC levels in a schizophrenia population versus a control patient population, and the specification teaches that for this population the difference is a 2.25 fold increase, the specification does not support the assertion in the some of claims that observing such an increase relative to any and all control populations of 2 or more individual is sufficient to suggest schizophrenia is present. Thus, given the lack of teaching in the specification and the highly unpredictable nature of the technology, an extensive amount of work would be required to practice the claimed invention.

In order to practice the claimed invention, one would have to undertake an extensive amount of experimentation in a highly unpredictable technology area. One would have to begin by validating the results observed in the instant specification in a separate population of healthy and schizophrenic patients, in view of the established level of unpredictability in this technology area. One would have to further complete similar analysis for other diseases and conditions and control populations versus healthy controls and versus schizophrenic controls in order to attempt to establish when and if analysis of CLC expression is sufficient to suggest schizophrenia is present. For example, consider the comparison of a test result and a control population of

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individuals with manic depression. If the test result is different from the level of expression observed in the manic depression control group, does this mean schizophrenia is present? Or if the control population is healthy individuals, how different from the average level of expression of healthy individuals would the test result have to be to indicate schizophrenia- is a 2.25 fold difference required or a higher or lower threshold? Some of the claims recite that any higher difference is sufficient or at least two times fold. The specification does not provide sufficient data for one of skill in the art to know what level is sufficient. Would any difference, up or down regulation be indicative of schizophrenia? Or could one result indicate schizophrenia and one a different disease such as RSV? Is CLC expressed in the blood of individuals with a disease other than schizophrenia or RSV? Is this expression also suggestive of other mental illnesses or other disorders entirely unrelated to schizophrenia? In order to reliably use a method for detecting schizophrenia, one would first have to answer at least these questions. One would also, however, have to carry out this testing for validation, for it is possible that the result observed in the instant specification is intrinsic to the cohort of patients evaluated in applicant's study. Further, one would have to undertake experimentation to determine difference thresholds required to determine that a patient has or does not have a disease.

As discussed, this art area is highly unpredictable.

## **Conclusion**

The claims include methods which encompass the detection in blood of the expression of CLC in a test subject and comparing this expression to control subjects, wherein the results are used to "classify the expression" or to suggest that an individual is a candidate for having schizophrenia. The identification of gene differential expression/disease indication relationships

is a highly unpredictable endeavor, requiring extensive experimentation. The specification provides minimal guidance. In light of the factors discussed, therefore, it is concluded that it would require undue experimentation to practice the claimed invention.

Although some of claims are drawn to a method of "detecting expression" or "classifying expression," and not to diagnosis or identifying increased likelihood of disease or the like, it is critical to understand how the classification can be used in order use the claimed invention. In this case, the specification does not provide sufficient guidance as to how to use the detecting or classification methods, or in other words what is the meaning of classifying expression "with that of subjects having" liver cancer or with subjects who are healthy? While one could do the method steps as written, thus satisfying the "how to make" aspect of 112 1st paragraph, the specification does not provide sufficient disclosure to satisfy the how to use aspect of the requirement.

Claim 58 represents a very narrow embodiment of the claimed invention, but still is based on data that is not replicated. As discussed in the rejection, it is established that the technology on which the instant claims is based is a highly unpredictable technology, and in the face of such a high level of unpredictability, replication is necessary before results can be considered sufficient to support claims directed at classifying the gene expression of an individual test subject. Therefore, even this claim, after having considered all of the factors set forth in this rejection, lacks proper enablement.

***Claim Rejections - 35 USC § 103***

5. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

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(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

6. Claims 52, 53, 54, 57, 58, 60, 61, 62, 63, 71, 72, 73, 75, 76, 77, and 78 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kuromitsu et al. (Gene Expression Patterns 1(2001) 17-21) in view of Sharma et al. (WO 98/49342).

Kuromitsu et al. teach a method for detecting expression of CLC gene in a human test subject suspected of having schizophrenia comprising detecting RNA encoded by said gene in said subject using an oligonucleotide of predetermined sequence which is specific for RNA encoded by said gene and/or for cDNA complementary to RNA encoded by said gene.

In particular Kuromitsu et al. teach using DNA chip expression analysis using the Hu6800 chip provided by Affymetrix. This chip inherently has thereupon probes for the analysis of CLC expression, and by using this chip they inherently tested for and detected any expression of this gene. This chip also inherently has thereupon housekeeping genes that are used for quantifying expression. In this rejection the "subject suspected of having schizophrenia" is any one of the test subjects who has schizophrenia. Expression that was at least two fold different was classified as differently expressed thus, the test subjects' expression was classified as that of a person having schizophrenia versus healthy controls when there was a two fold difference (p. 18) and if the p value was  $<0.05$  (p. 19). These ranges include the values set forth in claim 76, and thus if they were detected by Kuromitsu et al. they would be considered to indicate differentially expressed genes based on the express teachings of Kuromitsu et al.

Kuromitsu et al. do not teach detecting applying their analysis to the gene expression in a blood sample, and in particular detecting CLC in a blood sample.

Sharma et al. teach that from the very early stages of diseases the whole organism response to the changed condition (p. 10, 4<sup>th</sup> full ¶). In light of this, Sharma et al. teach a method for identifying a marker useful for diagnosing a disease comprising the steps of detecting the presence of RNA in an unfractionated sample of whole blood from each of one or more subjects having said disease and quantifying a level of said RNA in said sample. Namely, Sharma et al. teach the preparation of gene transcript patterns beginning with extraction of mRNA from tissues, cells or body parts of an individual or organism which has a disease or condition (p. 7, final ¶, p. 12, 1<sup>st</sup> ¶), and particularly teach the isolation of mRNA from unfractionated whole blood samples, where unfractionated is interpreted as meaning that the cell types within blood were not separated from one another (p. 35, section 5.1.1). Sharma et al. teach quantifying the level of expression and determining a difference between the quantified level in the sample from the diseased subject and a similarly quantified level of genes of control RNA from an unfractionated sample of whole blood from each of one or more first control subjects (p. 5, step (d); p. 15, first full ¶; p. 18, step (f); p. 11, final ¶). Sharma et al. teach that these methods are carried out by producing amplification products from RNA extracted from an unfractionated sample of whole blood (p. 18 and p. 35, Example 5). Sharma et al. specifically suggest that this method can be applied to the study of schizophrenia (p. 6, 3<sup>rd</sup> ¶).

Therefore, it would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to have modified the methods taught by Kuromitsu et al. so as to have additionally tested the blood of the patients having schizophrenia and the healthy control samples. One would have been so motivated by the express teachings of Sharma et al. that disease exerts a global effect on individuals and that this effect can be measured by gene

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expression in the blood. The identification of markers for disease in the blood suggests a potential minimally invasive method to detect this disorder of the brain- an organ whose sampling is very dangerous for patients. One would have been motivated to continue to use the microarray analysis taught by Kuromitsu et al. since they teach that this new technology "enables large-scale coordinated monitoring of gene expression" in an "unprecedented" fashion (p. 17).

**Response to Remarks**

The rejection under 112 1st paragraph has been amended to address the amended and newly added claims. Applicant traverses the rejection insofar as it applies to the pending claims, beginning on page 14 of the response.

Applicant states that the instant claims recite three clearly defined sets of controls. Not all claims are so limited. In addition, "patients that do not have schizophrenia" is sufficiently broad so as to encompass patients with all of the other diseases discussed in the rejection.

Applicant states that the newly added claims specify a direction and a level of difference in CLC expression to be detected. Applicant refers specifically to claim 75 which is not included in the enablement rejection. (It is noted that the limitation quoted as being in claim 75 on page 15 of the response is not actually in claim 75.) Nonetheless, as noted in the enablement rejection, not all of the claims are so narrowly limited. Further, even if the claims were all narrowly limited to mirror the relationships set forth in the specification, in a technology area of such a high degree of unpredictability, external validation would be extremely useful to establish the reliability of the observed relationships. In this case, very small sample sizes were used to establish the relationships set forth in the claims.

Applicant states that the fact that the disclosure of many other differentially expressed genes between healthy patients and those with schizophrenia does not detract from the gene in a biomarker for schizophrenia, but provides no further argument or evidence to support this assertion. These are two factors in a compilation of factors that lead to the conclusion of undue experimentation. Applicant points out that the specification discloses that RNA encoded by the CLC gene is 2.25 times higher than that of healthy subjects with a p value = 0.0212. This is acknowledged and discussed in the rejection. The specification also suggests that this gene is not expressed differently in patients who have manic depressive syndrome and schizophrenia. It is highly unpredictable as to whether that expression is the same or different as for other diseases.

Applicant contends on page 16 of the response that one of skill in the art can reasonably predict with statistical significance the probability that a patient may be a candidate for schizophrenia based on the teachings of the specification since the specification teaches the statistically significant correlation between levels of CLC RNA in blood of a diseased versus healthy controls. At the time the invention was made, the need for validation of such a result obtained by a microarray analysis was well understood by one of skill in this technology. The issues regarding the unpredictability of this technology are discussed in detail in this office action.

Applicant points out that the office action cites the larger study by Tsuang et al. which indicates that CLC RNA displays a statistically significant increase in expression in blood samples of schizophrenia patients versus healthy controls, and because the post-filing reference confirmed the showing in the specification, it is well within normal skill in the art to practice the

claimed invention. The post-filing date art cannot be used as evidence to establish that the specification was enabling at the time of filing.

Applicant states that Fjaerli et al. teach that CLC is downregulated in whole blood of infants hospitalized with RSV, and that the instant invention is that CLC is upregulated in schizophrenia patients. However, all of the claims do not include this recitation.

Applicant points out that neither Iwamoto et al., Tsuang et al. nor Vawter et al. contradict the relevant teachings of the specification. These references are not cited to contradict the teachings of the specification, but instead to illustrate the highly unpredictable nature of the technology area. The examiner is not stating that the results obtained in the instant specification are invalid. The technology area of this invention is highly unpredictable, with often discordant results being observed. The instantly disclosed results have not been validated, and as discussed by Tsuang et al. these results must be interpreted with caution. The results have not been validated in an external sample. Applicant states on page 17 that Iwamoto et al.'s statement that expression profiling is one of the strongest methodologies to reveal the molecular basis of mental disorders supports applicant's position that the instantly claimed invention should be presumed enabled. However, in the quotation from the reference is not addressing the state of the art at the time the invention was made, nor is it stating that all expression results should be taken as being enabled for detection of disease. To the contrary, the reference discusses many limitations with expression analysis that existed years following the filing of the instant patent application and highlights the unpredictable nature of the technology area.

Applicant points out that Iwamoto et al. does not suggest that experimental results obtained from blood samples are invalid. Nor does the examiner. Given the unpredictable

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nature of the technology at the time the invention was made, given the small sample sizes, given the fact that the results were not replicated, and given the other factors discussed in the rejection, the examiner maintains that there is not sufficient evidence on the record to support the enablement of the claimed invention.

Regarding Vawter et al., submits that they support the validity of the data in the instant specification (respose page 17). While recognizing the value of data obtained by microarray analysis, Vawter et al. also point out as a deficiency of their study that "we did not confirm all gene expression changes observed by microarray with real-time PCR," pointing out that genes which are differentially expressed by microarray may fail to replicate and that some cross-reactivity of cDNA families may be observed by such methods (p. 49, 1<sup>st</sup> column). While it is agreed that gene expression profiling is a strong methodology to reveal the molecular basis of mental disorders, this does not mitigate or remove the art recognized importance of validating and replicating data.

Applicant submits that the teachings of Tsuang et al. are clearly in favor of experimental data similar to that as disclosed as being reliable (p. 18), pointing to Tsuang et al. where they suggest that the work demonstrates the **potential** utility of blood-based RNA profiling in diagnostics (emphasis mine). But Tsuang et al. specifically teach that to validate their results and overcome the limitations of sample size and inferential errors, their "approach must be extended to larger extensively characterized sample sets, and the convergence of several lines of evidence will ultimately determine the reliability and usefulness of the identified putative biomarker genes..." and that "future investigations will be performed on drug-naïve patients or their non-psychotic first-degree relatives (p. 4)." Tsuang et al. cannot be mistaken as suggesting

that even the work they did was sufficient to establish the use of a single differentially expressed gene as sufficient to detect the presence of schizophrenia.

Applicant states that the results of Cheung et al. cannot be reliably extrapolated to primary blood samples since Cheung et al. are using cultured cell lines. However, this is irrelevant to the point of Cheung et al. which is that among individuals (in this case cell lines) there is natural variability in gene expression for any particular gene. Attorney arguments are not sufficient to establish that this biological fact is not the case.

The rejection is maintained and modified to address the amended claims.

7. The prior art made of record and not relied upon is considered pertinent to applicant's disclosure.


8. Shalit et al. (J Allergy Clin Immunol, Vol. 98, No. 2, August 1996) teach the detection of CLC in blood cells using gene specific primers. They fail to teach the expression of the blood of individuals suspected of having schizophrenia. Thus, since independent claim 58 requires that the test subject is "suspected of having schizophrenia" the population of potential subjects is limited by this recitation and Shalit et al. do not anticipate the instantly claimed invention. It is noted, however, that the instant disclosure is not the first evidence that the mRNA of this particular gene was present in human blood.

### ***Conclusion***

9. No claim is allowed.

10. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

11. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Juliet C Switzer whose telephone number is (571) 272-0753. The examiner can normally be reached on Monday, Tuesday, or ~~Thursday~~ <sup>Wednesday</sup>, from 9:00 AM until 4:30 PM. 

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached by calling (571) 272-0735.

The fax phone numbers for the organization where this application or proceeding is assigned are (571) 273-8300. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (571)272-0507.

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viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent application status and history information. It also enables applicants to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

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Juliet C. Switzer  
Primary Examiner  
Art Unit 1634

December 31, 2007